



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C07K 14/705**

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Joyce, J. [/]; (). DIETSCHY, John, M. [/]; (). TURLEY,  
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(54) Title: COMPOSITIONS AND METHODS OF MODULATING CHOLESTEROL METABOLISM

(54) Titre: COMPOSITIONS ET TECHNIQUES DE MODULATION DU METABOLISME DU CHOLESTEROL

(57) Abstract

The present invention relates to compositions and methods for reducing cholesterolemia and its effects. More specifically, the invention is directed, in one embodiment, to methods for screening for compounds that affect cholesterol levels generally, and in particular, that affect the absorption of cholesterol. The invention also is directed to methods of screening for compounds that increase bile acid synthesis. In so doing, the inventors describe useful transgenic cells and animals which lack one or both alleles of the LXR'alpha' gene. Also provided are therapeutic methods designed to reduce cholesterol levels in suitable subjects. The reduction may be effected by decreasing cholesterol absorption, increasing bile acid synthesis, or combinations thereof. Particularly useful in decreasing cholesterol absorption are RXR agonists, for example, rexinoid compounds. Therapeutic intervention in cholesterol biosynthesis and diet are additional adjunct therapies. In addition, the present invention relates to candidate compounds that modulate the expression of ABC-1 in a cell that expresses RXR. Methods of identifying and making a modulator of ABC-1 are disclosed.

(57) Abrégé

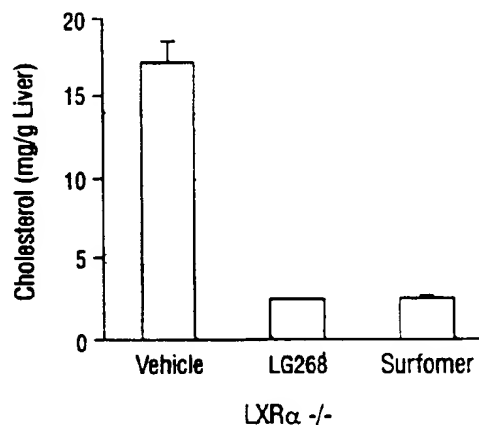
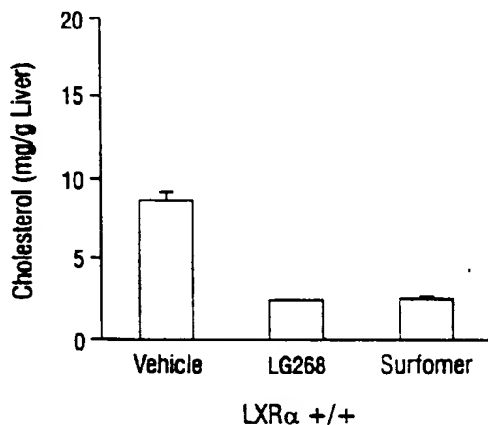
La présente invention concerne des compositions et des techniques de réduction de la cholestérolémie et de ses effets. Plus spécifiquement, l'invention concerne, dans un mode de réalisation, des techniques de sélection de composés qui affectent les taux de cholestérol en général, et en particulier, l'absorption de cholestérol. L'invention porte également sur des techniques de sélection de composés qui augmentent la synthèse acide biliaire. L'invention porte ainsi sur les cellules et les animaux transgéniques utiles manquant d'un ou des deux allèles du gène LXR'alpha'. L'invention se rapporte en outre à des techniques thérapeutiques destinées à faire baisser les taux de cholestérol chez des sujets indiqués. Cette baisse peut se produire grâce à une réduction de l'absorption de cholestérol, à une augmentation de la synthèse acide biliaire ou à une combinaison des deux. Les agonistes RXR, par exemple les composés réxinoïdes, sont particulièrement utiles dans la réduction de l'absorption de cholestérol. L'intervention thérapeutique dans la biosynthèse de cholestérol et un régime sont des thérapies additionnelles complémentaires. De plus, la présente invention concerne des composés candidats modulant l'expression de ABC-1 dans une cellule qui exprime un RXR. L'invention présente enfin des techniques d'identification et de création d'un modulateur de ABC-1.



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| (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application<br>US 60/111,894 (CON)<br>Filed on 10 December 1998 (10.12.98)   |  | Published<br>With international search report.   |  |
| (71) Applicant (for all designated States except US): BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West Seventh Street, Austin, TX 78701 (US).  |  | (88) Date of publication of the international search report:<br>9 November 2000 (09.11.00)   |  |
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## (54) Title: COMPOSITIONS AND METHODS OF MODULATING CHOLESTEROL METABOLISM



## (57) Abstract

The present invention relates to compositions and methods for reducing cholesterolemia and its effects. More specifically, the invention is directed, in one embodiment, to methods for screening for compounds that affect cholesterol levels generally, and in particular, that affect the absorption of cholesterol. The invention also is directed to methods of screening for compounds that increase bile acid synthesis. In so doing, the inventors describe useful transgenic cells and animals which lack one or both alleles of the LXR $\alpha$  gene. Also provided are therapeutic methods designed to reduce cholesterol levels in suitable subjects. The reduction may be effected by decreasing cholesterol absorption, increasing bile acid synthesis, or combinations thereof. Particularly useful in decreasing cholesterol absorption are RXR agonists, for example, rexinoid compounds. Therapeutic intervention in cholesterol biosynthesis and diet are additional adjunct therapies. In addition, the present invention relates to candidate compounds that modulate the expression of ABC-1 in a cell that expresses RXR. Methods of identifying and making a modulator of ABC-1 are disclosed.

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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/29497

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 C12N15/00 A01K67/027 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A01K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| X          | PEET DJ, TURLEY SD, MA W, JANOWSKI BA, LOBACCARO JM, HAMMER RE, MANGELSDORF DJ:<br>"Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha."<br>CELL 1998 MAY 29;93(5):693-704,<br>vol. 93, no. 5, 29 May 1998 (1998-05-29),<br>pages 693-704, XP000892013<br>cited in the application<br>the whole document<br>--- | 1-14, 44,<br>46       |
| A          | WO 96 21726 A (SALK INST FOR BIOLOGICAL<br>STUDI ; REGENTS BOARD OF (US))<br>18 July 1996 (1996-07-18)<br>claims 15-18; examples 6,7<br>---   | 14                    |
|            | -/--  |                       |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

10 May 2000

Date of mailing of the international search report

09.08.00

Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 99/29497

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| A          | <p>WILLY PJ, MANGELSDORF DJ.: "Unique requirements for retinoid-dependent transcriptional activation by the orphan receptor LXR."<br/> GENES DEV.,<br/> vol. 11, no. 3,<br/> 1 February 1997 (1997-02-01), pages<br/> 289-298, XP000905096<br/> the whole document</p> <p>---</p>  | 1                     |
| A          | <p>JANOWSKI ET AL: "An oxysterol signalling pathway mediated by the nuclear receptor LXR.alpha"<br/> NATURE,GB,MACMILLAN JOURNALS LTD. LONDON,<br/> vol. 383, no. 6602,<br/> 24 October 1996 (1996-10-24), pages<br/> 728-731-731, XP002104644<br/> ISSN: 0028-0836<br/> cited in the application<br/> the whole document</p> <p>-----</p> | 1                     |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/29497

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 21-29, 44-45

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## 1. Claims: 1-14 21-29 44-45

A non human transgenic mammal, the cells of which comprise at least one non functional endogenous LXRalpha allele;  
methods for screening a candidate substance using said mammal; a transgenic cell which comprises at least one non functional endogenous LXRalpha alleles;

## 2. Claims: 15-20

A method for screening an RXR agonist or LXRalpha agonist candidate substance for the ability to increase bile acid synthesis comprising :  
a) providing a cell;  
b) contacting said cell with said candidate substance; and  
c) monitoring a bile acid-related phenotype of said cell as far as not covered by the previous subject;

## 3. Claims: 30-33 46-47

A method for screening a rexinoid for the ability to inhibit cholesterol absorption by an intestinal cell comprising :  
a) providing an intestinal cell;  
b) treating said cell with said rexinoid; and  
c) monitoring cholesterol absorption by said cell as far as not covered by the previous subjects;

## 4. Claims: 34-43

A method of reducing cholesterol levels in a mammal comprising the step of treating said mammal with an RXR agonist; a method for inhibiting cholesterol absorption in a mammal comprising the step of treating said mammal with an RXR agonist;

## 5. Claims: 48-58

A method of screening for a modulator of ABC1 expression comprising :  
a) providing a cell expressing an RXR;  
b) contacting said cell with said rexinoid and candidate substance; and  
c) determining the expression of ABC1 in said cell; as far as not covered by the previous subjects;

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 99/29497

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| WO 9621726 A                              | 18-07-1996          | US 5747661 A               | 05-05-1998          |
|   |                     | AU 4686396 A               | 31-07-1996          |
| -----                                     |                     |                            |                     |